3 INTRODUCTION TO DNA PCR-BASED TYPING

The original PCR method, invented by Kary Mullis, was used to diagnose prenatal sickle-cell anemia.[1,3] Since the first report in 1985 of DNA amplification using the process known as polymerase chain reaction (PCR), many advances and modifications have been made to the basic method. PCR is an *in vitro* method for the enzymatic synthesis of specific DNA sequences, using two oligonucleotide primers that hybridize to opposite strands and flank the region of interest in the target DNA. A repetitive series of cycles involving template denaturation, primer annealing, and extension of the annealed primers by DNA polymerase results in the exponential accumulation of a specific fragment whose termini are defined by the 5' ends of the primers. Because the primer extension products synthesized in one cycle can serve as a template in the next, the number of target DNA copies approximately doubles at every cycle. Thus, 20 cycles of PCR yields about a million-fold amplification. With advances in the polymerase chain reaction process, analyzing forensic casework has been made more efficient. The ability to extract and type DNA from forensic evidentiary samples has revolutionized the field of forensic DNA. Previously, genetic marker typing was limited to the analysis of blood group markers and soluble polymorphic protein markers. Because the number of suitable markers expressed in particular fluids and tissues is relatively small, and because mixtures of fluids cannot be separated for conventional genetic marker typing, a suspect frequently cannot be included or excluded as a fluid donor in a case. However, the development of methods to extract DNA from virtually all biological specimens has greatly expanded the potential for individual identification.[3]

3.1. *Taq* DNA Polymerase

Initially, PCR amplification used the Klenow fragment of *E. coli* DNA polymerase I to extend the annealed primers. This enzyme was inactivated by the high temperatures required to separate the two DNA strands at the outset of each PCR cycle. Fresh enzyme had to be added constantly during every cycle. To make the process more time efficient, molecular biologists began to use an enzyme from a thermophilic eubacterial microorganism, *Thermus aquaticus* (*Taq*), capable of growth at 70-75^{0}C.[1,2] Although *Taq* DNA polymerase has a very limited ability to synthesize DNA above 90^{0}C, the enzyme is relatively stable and is not denatured irreversibly by exposure to high temperatures. Preliminary results indicated retention of 65% activity after a 50-cycle PCR amplification when the upper limit temperature was 95^{0}C.[1] Under normal reaction conditions, the amount of *Taq* DNA polymerase becomes limiting after 25-30 cycles of amplification.[4] Two of the forms of *Taq* DNA polymerase that are now available are genetically engineered forms of the native enzyme synthesized in *E. coli.* One of these DNA polymerases, AmpliTaq Gold™ (PE Applied Biosystems), is provided in an inactive state. Heat activates the enzyme. This feature allows flexibility in reaction setup. Both forms of the polymerase carry a 5'→3' polymerization-dependent exonuclease activity, but they lack a 3'→5' exonuclease activity.[4,5]

3.2. PCR Primers

In order to amplify a sequence of DNA, oligonucleotide primers flanking the target DNA sequence of interest are used. These oligonucleotides typically have different sequences and are complementary to sequences that lie on opposite strands of the template DNA with their 3' ends oriented toward each other. The template DNA is first denatured by heating in the presence of a large molar excess of each of the two oligonucleotides and the four dNTPs. The reaction is then cooled to a temperature that allows the oligonucleotide primers to anneal to their target sequences, after which the annealing primers are extended with the enzyme *Thermus aquaticus* (*Taq*) DNA polymerase in an automated series of heating and cooling cycles. With each cycle the DNA will approximately double the number of copies. The primers are responsible for the sequence specificity of the PCR reaction. Typically the

length of the primers used in the PCR amplification should be at least 16 nucleotides and preferably 20 to 30 nucleotides and single stranded.[4]

REFERENCES

1. Gelfand, D. H. and Erlich, H. A., PCR Technology: Principles and Applications for DNA Amplification, Stockton Press, New York, New York, 1989.

2. Engelke, D. R., Krikos, A., Brick, M. E., and Ginsburg, D., "Purification of *Thermus aquaticus* DNA Polymerase Expressed in *Escherichia coli*," Analytical Biochemistry, Vol. 191: 396-400, 1990.

3. Reynolds, R. and Sensabaugh, G., "Analysis of Genetic Markers in Forensic DNA Samples Using the Polymerase Chain Reaction," Analytical Chemistry, Vol. 63: 1-15, 1990.

4. Sambrook, J., Fritsch, E. F., and Maniatis, T., Molecular Cloning: A Laboratory Manual, Vol. 2, Cold Spring Harbor Laboratory Press, New York, 1989.

5. Longley, M. J., Bennett, S. E., and Mosvaugh, D. W., "Characterization of the 5'→3' Exonuclease Associated With *Thermus aquaticus* DNA Polymerase," Nucleic Acid Research, Vol. 18: 7317-7322, 1990.

GENERAL DNA STUDY QUESTIONS:

1. What is DNA?
2. Where is DNA found?
3. What is the role of DNA in the field of forensics?
4. What is the structure of DNA?
5. What is the composition of DNA?
6. What is a nucleotide?
7. What is a purine?
8. What is a pyrimidine?
9. What type of bonds holds the chains together?
10. What is complementary pairing?
11. What is a chromosome? gene? codon?
12. How many base pairs are there in a DNA molecule?
13. When would you expect two individuals to have the same DNA profile?

14. What is an allele?

15. What is meant by a genotype? a phenotype?

♦END